Allozyme analysis of a known hybrid zone between *Hyalophora euryalus* and *H. columbia gloveri* (Lepidoptera:Saturniidae) in the California Sierra Nevada.

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Abstract. Allozyme analysis of a hybrid zone between the moths *Hyalophora euryalus* and *H. columbia gloveri* confirmed the hybrid nature of populations previously shown (Collins, 1984) to be phenotypically intermediate and highly variable. Nine of 20 loci examined were polymorphic, and one locus (GAPDH) was fixed for alternate alleles in the two species, but heterozygous in the hybrid zone. Geographic patterns of allele frequencies conformed to expectation based on population genetic models. Several "rare alleles", unique to the hybrid zone, were found, as has often been seen in other hybrid zone studies.

Key Words: allozyme, *Hyalophora*, hybrid zone, hybridization

Introduction

Hybrid zones have played an important role as natural laboratories in speciation studies, especially since the advent of enzyme electrophoresis and other molecular techniques in systematics (Collins, 1991; Harrison, 1990, 1993; Hewitt, 1990). Two features of hybrid zones have intrigued evolutionary biologists: (1) the apparent stability and persistence of hybrid zones, and (2) the often abrupt phenotypic transition from intergrades into the parental populations bordering the hybrid zone. Hybrid zones are often distributed as long, narrow bands. Their geographic location and the coincidence of hybrid zones in diverse animal groups suggest that many hybrid zones are of post-Pleistocene origin, forming as previously isolated taxa invaded deglaciated regions and came into secondary contact (Harrison, 1970; Hewitt, 1990, Remington, 1968). The most widely accepted population genetic models of hybrid zones describe an equilibrium between gene flow, which would tend to widen the zone of intergradation, and selection against recombinant genotypes, which would tend to maintain sharp phenotypic boundaries (Barton and Hewitt, 1985; Endler, 1977). When the hybridizing

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Paper submitted 5 June 1995; revised manuscript accepted 19 December 1995.

taxa differ in critical developmental or reproductive traits recombinant genotypes will be inferior. Selection at these loci will impede gene exchange across the hybrid zone, thus preventing the fusion of the gene pools of the hybridizing taxa. Other neutral or beneficial alleles (at loci not closely linked to critical fitness loci) will introgress across the hybrid zone into the parental populations bordering the zone. Careful analysis of hybrid zones can reveal the genetic differences and similarities of closely related species, and thus shed light on the speciation process.

The failure of effective reproductive barriers to evolve in hybrid zones, in the face of hybrid unfitness, argues against the concept of reinforcement of mating barriers. If hybrid unfitness is severe, the very condition postulated to favor the origin of anti-hybridization mechanisms (Dobzhansky, 1970), the greater will be the barrier to introgression (Bigelow, 1965).

This paper describes a preliminary electrophoretic analysis of a hybrid zone between *Hyalophora euryalus* and *H. columbia gloveri* (Saturniidae) in the area of Monitor Pass, Alpine and Mono Co., California. Phenotypic variation and genetic compatibility among phenotypes has been thoroughly documented (Collins, 1984, 1991, unpubl.).

The frequency and geographic distribution of allozymes are especially useful in estimating the degree of gene flow across hybrid zones. Often the apparent genetic structure of hybrid zones as revealed by allozyme data differ from that expected based on distributions of morphological phenotypes (Harrison, 1990). We present preliminary allozyme data that suggest a high degree of concordance between these neutral genetic markers and morphology in these two species of *Hyalophora.*

Methods

All samples were collected in June 1994 using cylindrical moth traps with a fertile female as a pheromone source suspended in a small chamber above the trap funnel; pheromone response is not species-specific among the western *Hyalophora* (Collins, 1984). Representative samples of *Hyalophora euryalus* were collected in Nevada County, California. *Hyalophora columbia gloveri* were collected in Jefferson County, Colorado. Hybrid zone representatives were collected in Alpine County, California along Silver Creek at the base of Ebbetts Pass and in Lexington Canyon on the west side of Monitor Pass, and at a site on the east slope of Monitor Pass (Mono Co.). These sites represent a west to east transect across the hybrid zone (Figure 1).

Captured individuals were transported live in an ice chest and then stored until use in an ultra-cold freezer at -80°C. Isozyme variability was assayed at 20 presumptive loci (Table 1); general electrophoretic and histochemical staining methods followed Brussard et al. (1985) and May (1992). Genotype frequencies were obtained by direct count of phenotypes observed on the gels. The most common electromorph (allozyme) at each locus was designated as "C," with relatively faster migrating allozymes scored as "B" and still faster ones designated as "A." Likewise, allozymes that migrated slower than "C" alleles were scored as "D," and progressively later letters in the alphabet were assigned to still slower allozymes.

Estimates of heterozygosity, Nei's (1978) unbiased genetic distances and identities, and a UPGMA phenogram were made using BIOSYS-1 (Swofford and Selander 1981).

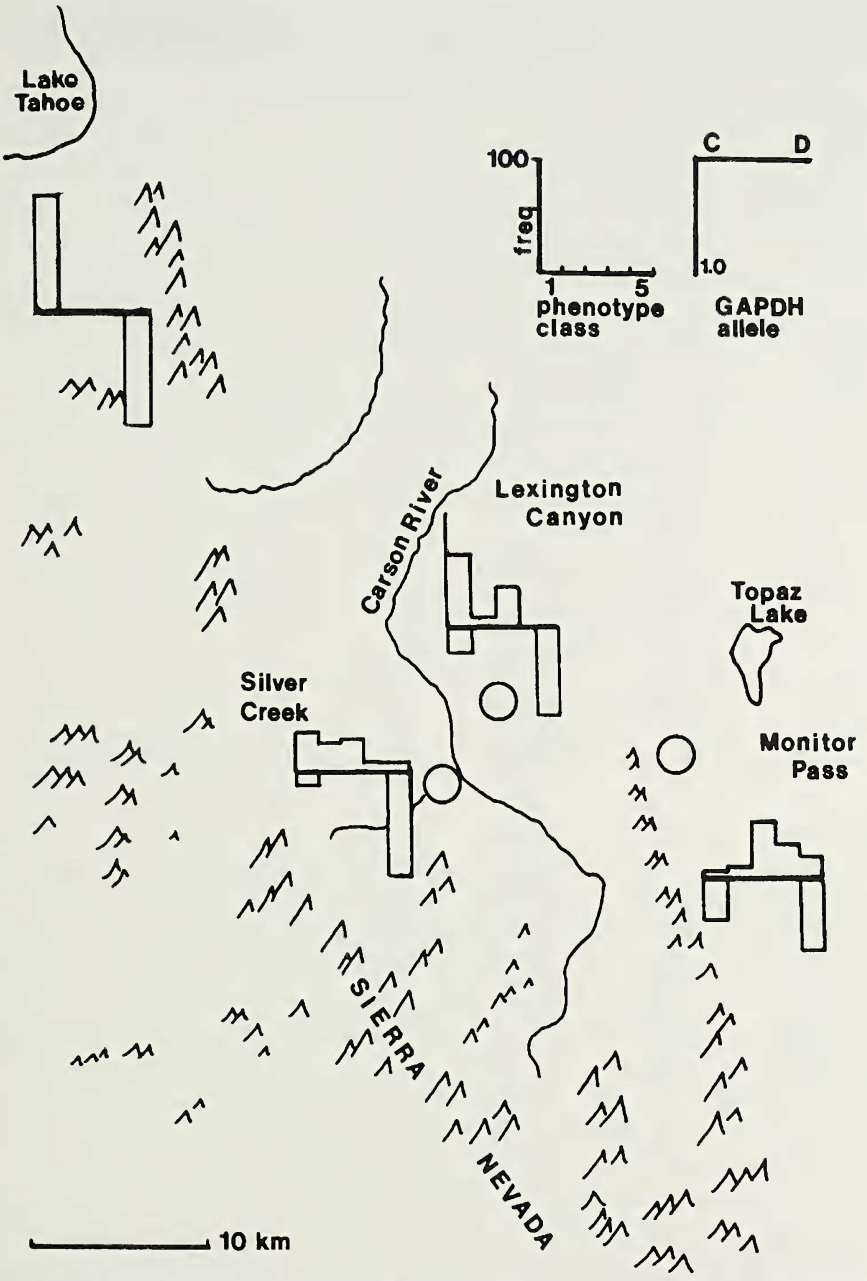

Figure 1. Map of *Hyalophora* hybrid zone, Alpine and Mono Co., California. Phenograms based on multivariate analysis of wing characters, with variation divided into five classes from pure *euryalus* (left) to pure *c. gloveri* (right) (Collins, 1984). The negative Y axis shows the frequencies of the "C" allele (fixed in *H. columbia gloveri*) and "D" allele (fixed in *H. euryalus*) of GAPDH. Collecting sites: Silver Creek (6500 ft.) at base of Ebbetts Pass; Lexington Canyon (5500 ft.) above Carson River; Monitor Pass (7300 ft.), east slope of Monitor Pass. Pure *H. euryalus* is found near Lake Tahoe, but due to introgression the nearest pure *H. columbia gloveri* is found only the isolated Panamint Mountains near Death Valley (not shown).

Table 1. Enzymes assayed and electrophoretic conditions used to analyze a hybrid zone between *Hyalophora euryalus* and *H. columbia gloveri* in the Sierra Nevada of California.

Locus	Enzyme	Enzyme Commission Number	Buffer
AAT-1,2	Aspartate aminotransferase	2.6.1.1	R
ALD	Aldolase	4.1.2.13	C
AK	Adenylate kinase	2.7.4.3	C
DDH	Dihydrolipoamide dehydrogenase	1.8.1.4	R
EST-F	Flourescent esterase	-.-.-.-	4
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	C
GPI	Glucosephosphate isomerase	5.3.1.9	R
GP-3,4	General protein	-.-.-.-	4
G3P	Glycerol-3-phosphate dehydrogenase	1.1.1.8	R
HA	Hexoseaminase	3.2.1.52	R
IDH	Isocitrate dehydrogenase	1.1.1.42	R
MDH-1,2	NAD Malate dehydrogenase	1.1.1.37	4
MPI	Mannosephosphate isomerase	5.3.1.8	R
MUP	Methylumbelliferyl phosphatase	-.-.-.-	C
PGDH	Phosphogluconate dehydrogenase	1.1.1.44	4
SOD	Superoxide dismutase	1.15.1.1	C
XDH	Xanthine dehydrogenase	1.1.1.204	R

Results

Sample sizes and allele frequencies are given in Table 2. Polymorphisms were detected at nine of the 20 assayed isozyme loci in at least one of the sampled populations (Table 2). A fixed allelic difference was detected at the GAPDH locus with *H. euryalus* fixed for the D allele and *H. columbia gloveri* fixed for the C allele (Table 2). Hybrid zone samples were found to be segregating for both alleles at GAPDH (Table 2). Similarly, *H. euryalus* segregated for alleles B and C at AK-1 while *H. c. gloveri* segregated for the alleles C and D at this locus (Table 2). The sample from Monitor Pass within the hybrid zone segregated for all three alleles (B,C, and D) at AK-1 (Table 2). Hybrid zone samples segregated for several alleles not detected in either parental species, e.g. AAT-1 B and D, AAT-2 B, IDH-1 D (Table 2).

Nei's (1978) unbiased genetic distances and identities for all pairs of samples are given in Table 3. Both of these estimates of overall genetic similarity exhibited an east to west cline in genetic similarity for *H. c. gloveri* from Colorado (Table 3). The *H. c. gloveri* sample was most similar to the Monitor Pass hybrid sample which was the eastern-most sample within the hybrid zone, and was least similar to *H. euryalus* sampled from west of the hybrid zone in California (Table 3). The *H. euryalus* sample was genetically more similar to the hybrid samples than it was to the *H. c. gloveri* sample (Table 3) and did not exhibit a directional decrease in genetic similarity

Table 2. Sample sizes and allele frequencies at polymorphic loci assayed in *Hyalophora euryalus* and *H. c. gloveri* including hybrid samples with expected mean heterozygosity estimates, H (S.E.).

		Hybrid Zone			*H. euryalus*	*H. c. gloveri*
Site		Monitor Pass	Lexington Canyon	Silver Creek	California	Colorado
Sample size		6	7	10	6	6
Locus	Allele					
AAT-1	B	0.000	0.214	0.000	0.000	0.000
	C	0.833	0.714	0.550	1.000	1.000
	D	0.167	0.071	0.450	0.000	0.000
AAT-2	B	0.000	0.071	0.000	0.000	0.000
	C	1.000	0.929	1.000	1.000	1.000
AK-01	B	0.167	0.000	0.000	0.111	0.000
	C	0.750	0.857	0.850	0.889	0.833
	D	0.083	0.143	0.150	0.000	0.167
GAPDH	C	0.417	0.214	0.056	0.000	1.000
	D	0.583	0.786	0.944	1.000	0.000
GPI-1	C	0.750	0.857	0.800	0.944	1.000
	D	0.250	0.143	0.200	0.056	0.000
IDH-1	B	0.000	0.000	0.000	0.063	0.000
	C	1.000	0.786	0.800	0.875	0.833
	D	0.000	0.214	0.200	0.063	0.167
MDH-1	B	0.000	0.000	0.050	0.000	0.000
	C	1.000	1.000	0.950	0.444	1.000
	D	0.000	0.000	0.000	0.556	0.000
MPI-1	B	0.083	0.000	0.000	0.000	0.000
	C	0.917	1.000	0.900	0.944	1.000
	D	0.000	0.000	0.100	0.056	0.000
PGDH	C	1.000	1.000	1.000	0.889	1.000
	D	0.000	0.000	0.000	0.111	0.000
H		0.092 (0.039)	0.093 (0.035)	0.093 (0.035)	0.070 (0.030)	0.030 (0.021)

Table 3. Unbiased genetic distances (Nei 1978) above diagonal and unbiased genetic identities (Nei 1978) below diagonal for *Hyalophora euryalus*, *H. c. gloveri*, and hybrid zone samples assayed at 20 presumptive isozyme loci.

	1	2	3	4	5
1. Monitor Pass	——	0.001	0.009	0.026	0.020
2. Lexington Canyon	0.999	——	0.002	0.021	0.033
3. Silver Creek	0.991	0.998	——	0.026	0.059
4. *H. euryalus*	0.974	0.980	0.974	——	0.070
5. *H. c. gloveri*	0.980	0.968	0.943	0.932	——

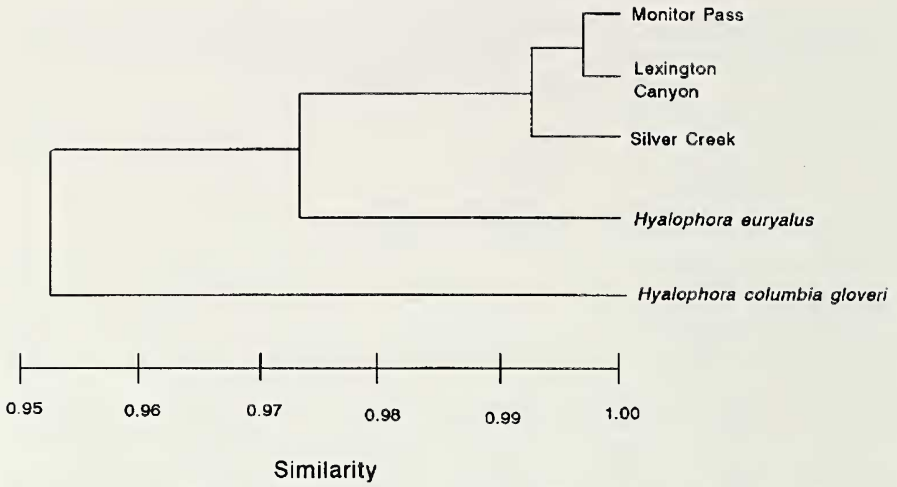

Figure 2. UPGMA phenogram based on Nei's (1978) unbiased genetic distances for 20 isozyme loci. The cophenetic correlation is 0.808.

across the hybrid zone as did *H. c. gloveri*. The UPGMA phenogram in Figure 2 shows the close affinity among the hybrid zone samples and the greater overall genetic similarity between the California *H. euryalus* sample with the hybrid zone samples than with *H. c. gloveri* collected in Colorado.

Estimated mean individual heterozygosity expected under Hardy-Weinberg assumptions ranged from 0.030 in the *Hyalophora columbia gloveri* sample to 0.093 in both the Lexington Canyon and Silver Creek samples (Table 2). Estimated heterozygosity was higher in the hybrid zone samples than in the parental populations (Table 2). Significant deviations from Hardy-Weinberg expectations were detected at four loci (AAT-1 in the Lexington Canyon and Silver Creek samples, AK-1 in the Silver Creek sample, and MDH-1 in the *H. euryalus* sample). All four significant deviations from expected Hardy-Weinberg genotypic proportions were heterozygote deficiencies. Given that only four percent, i.e. four of 100, of the tests performed for conformance to Hardy-Weinberg equilibrium indicated significant deviations, no significant trend in departures from Hardy-Weinberg expectations was detected.

In summary, the genetic data are completely consistent with the geographic distribution of the samples and the expected pattern of genetic exchange through the hybrid zone. A multivariate morphometric analysis of wing pattern traits in the hybrid zone (Collins, 1984) clearly revealed a west-to-east transition from *euryalus*-like to *c. gloveri*-like across Monitor Pass (Figures 1 and 3). Variability was highest in mid-hybrid zone, and introgression for certain wing traits, such as hindwing discal spot shape, showed evidence of introgression of *euryalus* genes into the *c. gloveri* populations bordering the hybrid zone and extending south along the east slope of the Sierra Nevada.

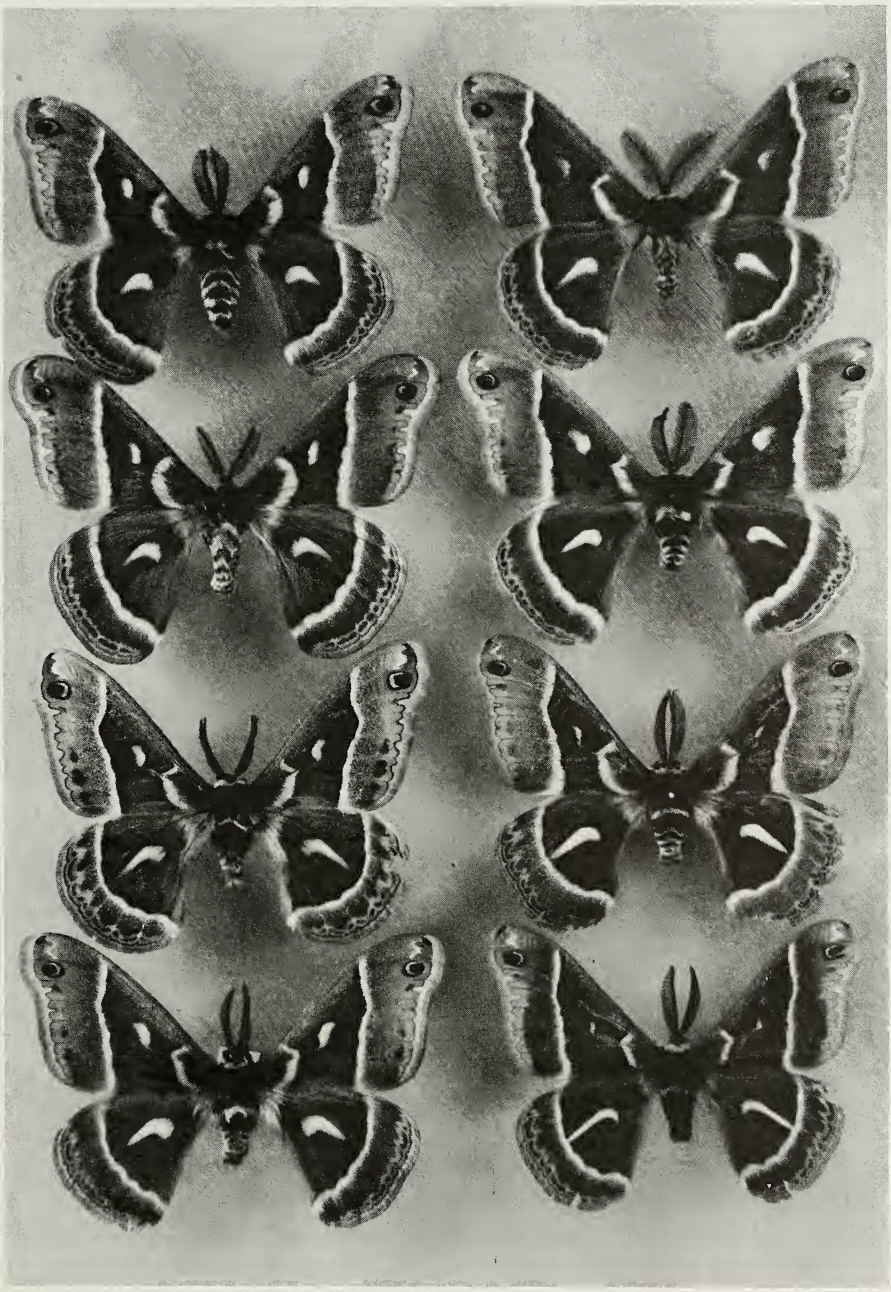

Figure 3. Range of *Hyalophora* phenotypes present in the Monitor Pass hybrid zone. Top left specimen resembles pure *H. columbia gloveri*; lower right specimen resembles pure *H. euryalus*. Other specimens indicate complete intergradation of characters otherwise diagnostic for the two species, such as hindwing discal spot shape, width of white bands, and coloration. All specimens wild males collected in funnel traps.

Discussion

Population genetic theory provides a set of expectations for the spatial pattern of allozyme variability within hybrid zones (Barton and Hewitt, 1985; Porter, 1990). Assuming geographically uniform effects of drift in local populations and no selection at loci under examination, frequencies of genetic markers are expected to be vary inversely with geographic distance, i.e. to be directly related to degree of effective gene flow. Overall heterozygosity should be higher in hybrid zones, and hybrid zone individuals are expected to be heterozygous at loci with fixed allelic differences in the parental populations. Finally, several allozyme studies of hybrid zones (Barton and Hewitt, 1985; Woodruff, 1989) have revealed the existence of unique electromorphs, the "hybrizymes" of Woodruff (1989), in hybrid zone samples that are not found segregating in the parental populations.

The allozyme data confirm the close genetic affinity of *Hyalophora euryalus* and *H. columbia gloveri* that is indicated by the morphological and ecological data (Collins, 1984). Furthermore, these data conform well to the genetic results expected for populations within a hybrid zone. *H. euryalus* and *H. c. gloveri* are genetically very similar with a genetic identity of 0.932 (Table 3). This estimate puts these two morphologically distinct species within the range of genetic identity estimates for subspecies or sibling species reported in similar studies of other Lepidoptera taxa. For example, Brussard et al. (1985) estimated a mean genetic identity of 0.964 between butterfly species in the sub-family Melitaeini using nearly identical techniques. Britten and Brussard (1992) found similar estimates of genetic identity between widely separated subspecies of *Boloria improba* in western North America. Likewise, Brittnacher et al. (1978) estimated a mean genetic identity of 0.977 for subspecies in the butterfly genus *Speyeria*. In a study of natural hybridization among western black swallowtails, Sperling (1987) found a genetic identity between *Papilio zelicaon* and *P. machaon oregonius* of 0.797 to 0.836 (depending on sample locality) and between *P. zelicaon* and *P. polyxenes* of 0.865. Hagen et al. (1991) found a genetic identity of 0.86 for the morphologically similar *Papilio glaucus* and *P. canadensis*. These taxa, previously thought to represent subspecies, are ecologically distinctive and are separated by a narrow hybrid zone with abrupt character clines.

Pairwise genetic identities also decrease from east to west through the hybrid zone for *H. c. gloveri* from Colorado (Table 3). These estimates were expected to show a similar decrease through the hybrid zone from west to east for *H. euryalus* but, the directional pattern of genetic identities is less clear in the hybrid zone for this parental species (Table 3). This asymmetry is consistent, however, with the morphological evidence for asymmetrical introgression of *H. euryalus* genes across the hybrid zone.

Allele frequencies in general do not show strong geographic trends through the hybrid zone (Table 2). For example, MDH-1 B has a frequency of 0.556 in the *H. euryalus* sample from the western side of the hybrid zone but was not segregating in the nearby Silver Creek sample taken from within the hybrid zone (Table 2). This may be an artifact of the relatively small number of individuals assayed for isozyme variability. As expected, heterozy-

gosity estimates are higher in the hybrid zone samples than for the parental samples (Table 2). The most compelling allozyme evidence for the existence of a hybrid zone for these moth species was found at the GAPDH locus. Fixed allelic differences were revealed at this locus for *H. euryalus* and *H. c. gloveri* (Table 2). Hybrid zone populations were heterozygous at GAPDH and segregated for both parental species' alleles at this locus (Table 2). Furthermore, allele frequencies changed directionally as predicted; for example, the frequency of GAPDH C, the "eastern" *H. c. gloveri* allele, declined from 0.417 in the Monitor Pass sample in the eastern part of the hybrid zone, to 0.214 at Lexington Canyon in the center of the hybrid zone, to 0.056 at Silver Creek in the western part of the hybrid zone (Table 2). This is the predicted pattern for loci with fixed allele differences in the parental species, and for directional changes in allele frequencies for polymorphic loci.

The existence of unique alleles (also called "rare alleles") found only in the hybrid zone and not in the parental samples (e.g. AAT-2 B, MDH-1 B, and MPI-1 B and D; Table 2) can be taken as evidence that a hybrid zone is present at the sampled localities. Several studies (reviewed in Barton and Hewitt, 1985 and Woodruff, 1989) have found similar unique electromorphs in samples taken from hybrid zones within a wide range of species. As noted by Woodruff (1989), the origin and maintenance of hybrid zone-specific electromorphs are difficult to explain. Woodruff (1989) favors a model of intragenic recombination at polymorphic loci that yields unique hybrid zone alleles, maintained at their relatively high observed frequencies by either selection (possibly acting at tightly linked loci), or by some form of genetic drift.

The allozyme data reported above confirm the presence of a relatively narrow hybrid zone between *H. euryalus* and *H. c. gloveri* in the Sierra Nevada as first described by Collins (1984, 1991) based on morphology and reproductive fitness. The origin and maintenance of this hybrid zone are the subject of ongoing investigations. An unusual feature of the zone is the high reproductive fitness of intergrade females within the zone, in contrast to the low fecundity of hybrid females from lab matings between parental phenotypes from stock from opposite sides of the hybrid zone. A similar regional optimization of genetic compatibility has been observed in an orthopteran hybrid zone in the Pyrenees (Virdee & Hewitt, 1994). With further analysis it may be possible to correlate some aspect of allozyme or mitochondrial DNA variation with the subdivision of the hybrid zone seen in the regional optimization of genetic compatibility described above. Porter (1990) used estimates of gene flow across a hybrid zone in *Limenitis* to evaluate the taxonomic status of the hybridizing pair. Similar techniques could be used when data are available to examine species boundaries among the *Hyalophora* which appear to vary greatly in degree of gene exchange in various contact zones (Tuskes et al., 1996).

Acknowledgements. The Nevada Biodiversity Initiative provided funding and facilities for electrophoretic and data analyses. An EPSCor Grant to V.R. provided partial support for the sample collection and laboratory aspects of this work. We are grateful

to Peter Brussard for his help in arranging this cooperative project. We thank Adam Porter, Arthur Shapiro, and an anonymous reviewer for their insightful comments.

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